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Research Article

In Vitro Leishmanicidal Activity of Different Chalcone, Phthalonitrile and Their Peripheral Tetra Zinc Phthalocyanine Derivatives

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Abstract

In this study, *in vitro* leishmanicidal activities of different chalcone compounds (**5-8**), phthalonitrile derivatives (**5a-8a**), and zinc phthalocyanine (**5b-8b**) complexes bearing chalcone compound in peripheral positions were investigated. Phthalonitrile derivatives were obtained in the reaction of 4-nitrophthalonitrile with chalcone compound obtained by the reaction of acetophenone and various aldehydes. Zinc phthalocyanine complexes containing chalcone in the peripheral position were obtained as a result of the reaction of the synthesized phthalonitrile derivative with $Zn(CH_3COO)_2$ metal salt. The characterization of the synthesized original compounds (**8**, **8a** and **8b**) was performed by various spectroscopic methods (IR, ^1H and ^{13}C NMR, MALDI-TOF-MS and UV-Vis). Leishmaniasis is a disease lead to by parasites of the genus *Leishmania*, which can result in death as well as various clinical syndromes, generally in developing countries. New drug studies are needed because the drugs used in the treatment are toxic and resistance develops against them. In this study, the leishmaniacidal activities of synthesized chalcone, phthalonitrile and phthalocyanine series substances against *Leishmania infantum* and *Leishmania major* parasites were evaluated for the first time.

Keywords: Chalcone, phthalocyanine, *leishmania promastigotes*, anti-lesichmania

Farklı Kalkon, Ftalonitril ve Periferal Tetra Çinko Ftalosiyanin Türevlerinin *In Vitro* Layşmanyasidal Aktivitesi

Öz

Bu çalışmada, farklı kalkon bileşikleri (5-8), ftalonitril türevleri (5a-8a) ve periferik pozisyonlarda kalkon bileşiği taşıyan çinko ftalosiyanin (5b-8b) komplekslerinin *in vitro* layşmanisidal aktiviteleri araştırıldı. Asetofenon ve çeşitli aldehitlerin reaksiyonu ile elde edilen kalkon bileşikleri ile 4-nitroftalonitrilin reaksiyonundan ftalonitril türevleri elde edilmiştir. Sentezlenen ftalonitril türevinin $Zn(CH_3COO)_2$ metal tuzu ile reaksiyonu sonucunda periferal pozisyonda kalkon içeren çinko ftalosiyanin kompleksleri elde edilmiştir. Sentezlenen orijinal bileşiklerin (8, 8a ve 8b) karakterizasyonu çeşitli spektroskopik yöntemlerle (IR, ^1H ve ^{13}C NMR, MALDI-TOF-MS ve UV-Vis) gerçekleştirilmiştir. Layşmaniasis, genellikle gelişmekte olan ülkelerde, Layşmania cinsi parazitlerinin neden olduğu, ölüme ve çeşitli klinik sendromlara neden olabilen bir hastalıktır. Tedavide kullanılan ilaçların toksik olması ve bunlara karşı direnç gelişmesi nedeniyle yeni ilaç çalışmalarına ihtiyaç duyulmaktadır. Bu çalışmada, sentezlenen kalkon, ftalonitril ve ftalosiyanin serisi maddelerin *Layşmania infantum* ve *Layşmania major* parazitlerine karşı layşmaniasidal aktiviteleri ilk kez değerlendirilmiştir.

Anahtar Kelimeler: Kalkon, ftalosiyanin, *layşmanyaz promastigotları*, anti-layşmanyaz aktivite

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1. Introduction

Leishmaniasis is an overlooked tropical parasitic disease found worldwide in tropical, subtropical and some countries of southern Europe. The disease still maintains its importance in developing countries. The disease is passed on humans by the bite of phlebotoma sand flies and has several different clinical forms [1]. The first form is visceral leishmaniasis (VL) can be fateful if left untreated. VL is diagnosed with weight loss, bouts of fever, anemia and spleen and liver enlargement. Most cases are detected in Brazil, India and East African countries. The most prevalent form of leishmaniasis is the cutaneous form, which causes skin lesions, primarily ulcers, on exposed parts of the body. The other form is mucocutaneous leishmaniasis, which usually affects the mucous membranes of the nose, mouth and throat in countries such as Bolivia, Brazil, Ethiopia and Peru (WHO). This disease is endemic in 98 countries and more than 350 million people are at risk of infection. It also causes 20000-40000 deaths worldwide each year [2]. There is no effective vaccine to prevent the disease. Pentavalent antimony compounds (SbV) are the first-choice drugs for the treatment of all types of leishmaniasis. In the treatment of the disease, drugs such as liposomal amphotericin B, azole groups, miltefosine and paromomycin are preferred as the second choice. Due to the high toxicity, cost and clinical resistance of existing drugs, new drug development against Leishmaniasis is needed [3]. In recent years, many studies have been carried out to develop drugs that are safe, inexpensive and have few side effects. For this purpose, antileishmanial tests were carried out on various synthetic and natural compounds [4,5]. In this work, it was aimed to researched the leishmanicidal activity of chalcone compounds against *Leishmania infantum* and *Leishmania major* promastigotes by microdilution broth method containing alamar blue.

Chalcone is an aromatic ketone possessing an α,β-unsaturated carbonyl group consisting of two aromatic rings. Chalcone derivative compounds have recently attracted the attention of researchers, especially in the departments of synthetic organic and pharmaceutical chemistry. Medicinal chemists interest in chalcone compounds because of their biological activity, which include anti-bacterial [6], antiobesity [7,8], anticancer [9], antioxidant [10,11], antileishmanial [12,13], anti-diabetic [14], antifungal [15], antiulcer [16], antitubercular [17], anti-inflammatory [18], antimalarial [19] and antiviral [20].

Phthalocyanines (Pcs), which have planar macrocyclic molecules and contain nitrogen atoms in the inner loop, contain a class of highly robust and versatile chemical compounds [21]. Phthalocyanines, which are still of great technological importance for the production of green and blue pigments, have applications such as such as DNA-binding and enzyme inhibition [22], catalysts [23,24], metal sensors [25], electrochemistry [26,27], solar cells [28], Langmuir-Blodgett films [29], fluorometric properties [25], liquid crystals [30], photodynamic therapy (PDT) [31], electrophotography [32], antimicrobial agent [33], optical disks [34], electrochromic displays [35], antioxidant [36] and modified supports in recent years. The leismania activity studies of phthalocyanines, which have been studied in many application

areas, have not been found in the literature before. Since phthalocyanines (especially zinc phthalocyanines) show activity especially in the biological field [22, 33, 36, 31], this study was designed due to it is a question of how they will show activity against *Leishmania* parasites.

For the purpose of the study, first of all, we synthesized different chalcone compounds (5, 6, 7 and 8) by thinking that chalcone compounds may be an active in biological studies because of the their medical applications in literature. In the second step, phthalonitrile derivatives (5a-8a), which are the starting compound for the synthesis of zinc phthalocyanine compounds were obtained the reaction of chalcone compounds with 4-nitrophthalonitrile. In the last part of the synthesis stage, the synthesis of peripheral tetra zinc phthalocyanine complexes (5b-8b) take place as a result of the reaction of the synthesized phthalonitrile derivatives with the zinc salt. The synthesized original compounds (8, 8a and 8b) were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, UV-Vis (for zinc pthalocyanine 8b) and mass spectrometry. In the last part of the study, leishmanicidal activity of the synthesized chalcone (5, 6, 7 and 8), phthalonitrile (5a-8a) and zinc phthalocyanine (5b-8b) compounds were investigated.

2. Methods and Material

The used materials were supplied as Supplementary Information.

2.1. Synthesis

2.1.1. (2E)-3-(5-ethylthiophen-2-yl)-1-(3-hydroxyphenyl)prop-2-en-1-one (8)

The new chalcone compound (8) was synthesized using the method in the literature [37,38,39]. You can find detailed information in Supplementary Information. The obtained red product was cleansed by column chromatography (SiO_2) method using mobile phase $CHCl_3$ (chloroform) solvent. The structure of the pure compound was characterized by spectroscopic methods. Yield: 1.61 g (44 %). IR (ATR) (ν, cm^{-1}): 3333 (O–H), 3068 (Ar–H), 2969-2873 (Aliph.–H), 1643 (C=O), 1558-1446 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 7.94-7.90 (d, *J*=15Hz, 1H, =CH); δ = 7.65 (s, 1H, Ar–H); δ = 7.57-7.55 (d, *J*=8Hz, 1H, Ar–H); δ = 7.40-7.36 (t, *J*=15, 1H, =CH); δ = 7.23-7.19 (m, 2H, Ar–H); δ = 7.14-7.12 (d, *J*=6Hz, 1H, Ar–H); δ = 6.81-6.78 (m, 1H, Ar–H), δ = 6.57 (s, 1H, O–H), δ = 2.90-2.88 (k, 2H, –CH_2), δ = 1.35 (t, 3H,–CH_3). ^{13}C NMR (400 MHz, $CDCl_3$, (δ) ppm): 190.20 (C=O), 156.46 (C–OH), 152.68, 139.67, 138.35, 137.94, 133.15, 129.83, 125.12, 120.76, 120.24, 119.36, 115.09, 23.99 (–CH_2), 15.56 (–CH_3). MALDI-TOF-MS (m/z) Found: 258.81 [M]$^{+}$.

2.1.2. 5-{3-[(2E)-3-(5-ethyl-2-thienyl)prop-2-enoyl]phenoxy}phthalonitrile (8a)

The new chalcone bearing phthalonitrile compound (8a) was synthesized using the method in the literature [37,38,39]. You can find detailed information in Supplementary Information. The dried product was cleansed by column chromatography using alumina (Al_2O_3) as column material and a $CHCl_3$:EtOH (5:1) solvent system as mobile phase. An oily brown pure product was obtained. Yield: 0.72 g (30 %). IR (ATR) (ν, cm^{-1}): 3071 (Ar–H), 2965-2855 (Aliph.–H), 2232 (C≡N), 1664 (C=O), 1578-1436 (C=C), 1385-1246 (Ar-O-Ar). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ= 8.03 (s, 1H, Ar–H); δ = 7.92 (d, *J*= 8Hz, 1H, Ar–H); δ= 7.78 (d, *J*= 8Hz, 2H, Ar–H); δ= 7.69 (s, 2H, Ar–H); δ= 7.63 (t, 2H, Ar–H); δ = 7.33 (m, 3H, =CH and $CDCl_3$); δ = 2.65 (s, 2H, –CH_2); δ = 1.27 (s, 3H, –CH_3). ^{13}C NMR (400 MHz, $CDCl_3$, (δ)ppm): 196.52

(C=O), 162.55 (ArC–O), 161.20 157.14, 154.14, 139.71, 135.55, 131.04, 126.20, 125.17, 125.12, 121.74, 121.68, 121.64, 119.92, 117.86 (–C≡N), 115.20 (–C≡N), 114.78, 109.60, 108.93, 108.50, 31.44, 26.71. MALDI-TOF-MS (m/z) Found: 381.13 $[M-3]^+$.

2.1.3. Zinc(II) phthalocyanine (8b)

The new chalcone substituted zinc phthalocyanine (8b) was synthesized using the method in the literature [37,38,39]. You can find detailed information in Supplementary Information. The resulting crude product was cleaned by column chromatography on alumina (Al_2O_3) by using $CHCl_3$:CH_3OH as the eluent system. Yield: 7 mg (14%). mp: >300 ^oC. IR (ATR) (ν, cm^{-1}): 3061 (Ar–H), 2.924-2854 (Aliph.–H), 1644 (C=O), 1599-1435 (C=C), 1359-1260 (Ar–O–Ar). ^1H NMR (400 MHz, DMSO-d_6 ppm) δ = 8.32 (s, 44 H, Ar–H), 3.39 (s, 8H, –CH_2), 1.62 (m, 12H, –CH_3). UV-Vis (DMF): λ_{max}, nm (log ε): 681 (5.02), 613 (4.56), 352 (5.16).

2.2. *Determination of in vitro Leishmanicidal Activities*

The antiparasitic properties of chalcone, phthalonitrile and their zinc phthalocyanine derivatives were defined by testing their leishmanicidal activities against *Leishmania infantum* and *Leishmania major* promastigotes. The studies were achieved *in vitro* using microdilution broth method containing the alamar blue. The used method and materials were given as Supplementary Information.

3. Results and Disscussion

3.1. *Synthesis and Characterization*

The compounds in the study and the original synthesized compounds were summarized in Figures 1 and 2. Chalcone derivatives bearing different groups (5, 6 and 7), phthalonitriles (5a, 6a and 7a) and their zinc phthalocyanine complexes (5b, 6b and 7b) were mentioned in our previous studies (Figure 3) [37-39].

The original chalcone compound bearing the thiophene group (8) was obtained by the Claisen-Schmidt reaction of 5-ethylthiophene-2-carbaldehyde (a) and 1-(3-hydroxyphenyl) ethanone (4) (aromatic ketone) (Figure 1).

The synthesis of the chalcone-based phthalonitrile derivative (8a) was accomplished by the nucleophilic substitution reaction between the chalcone compound (2*E*)-3-(5-ethylthiophen-2-yl)-1-(3-hydroxyphenyl)prop-2-en-1-one (8) and 4-nitrophthalonitrile (Figure 2). Synthesized phthalonitrile compound (8a) containing thiophene-chalcone group was used to synthesize zinc phthalocyanine compound (8b). The Zn(II)Pc (8b) was achieved from the reactions of 5-{3-[(2*E*)-3-(5-ethyl-2-thienyl)prop-2-enoyl]phenoxy}phthalonitrile (8a) with the metal salt $Zn(CH_3COO)_2$ in *n*-pentanol and DBU as the catalyst. After purification, spectroscopy techniques (IR, NMR and mass) were used to identify of the structure of chalcone (8), phthalonitrile (8a) and zinc phthalocyanine compound (8b) (Figures 4-7).

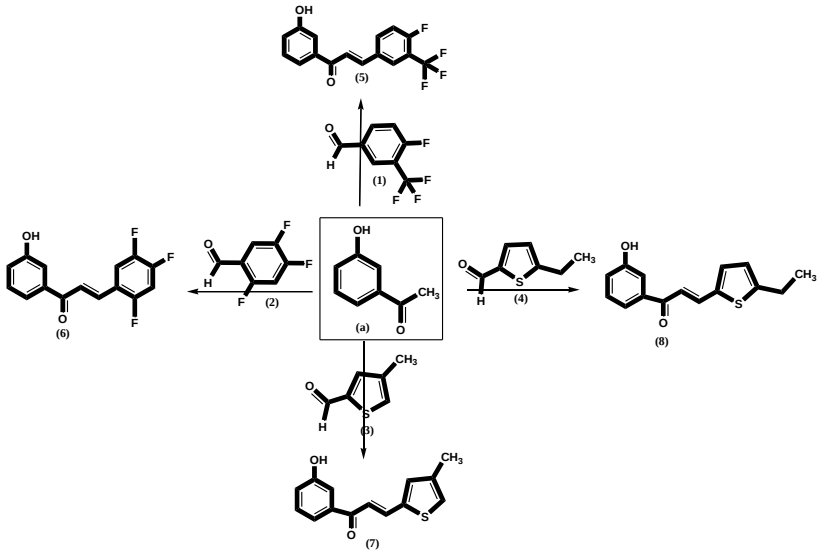

Figure 1. The synthetic route to the chalcone compounds (5) [37], (6) [39], (7) [38] and (8)

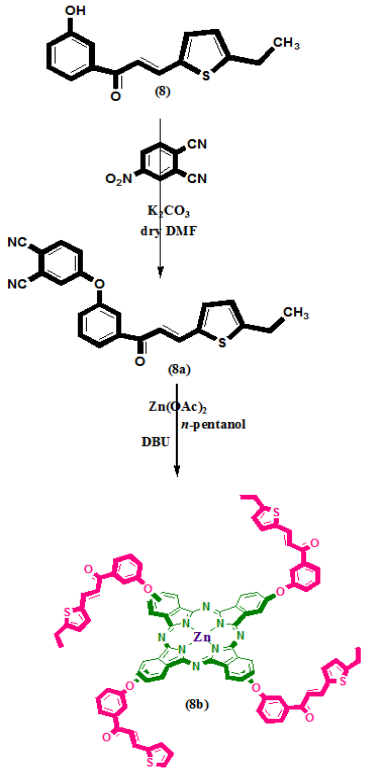

Figure 2. The synthetic route to the original phthalonitrile compound (8a) and zinc phthalocyanine (8b)

In the IR spectrum of chalcone compound (2*E*)-3-(5-ethylthiophen-2-yl)-1-(3-hydroxyphenyl)prop-2-en-1-one (8) , the bands were observed at 3333 cm^{-1} (OH stretching), 3068 cm^{-1} (aromatic CH stretching vibration), 2969-2873 cm^{-1} (aliphatic CH stretching vibration), 1643 cm^{-1} (C=O stretching) and 1558 cm^{-1} (Ar–C=C stretching vibrations). In the IR spectrum of (8a), the expected after nucleophilic substitution reaction, the nitrile strong

characteristic strecthing band at 2232 cm^{-1} (C≡N) was clearly observed. The band belonging to the OH group (hydroxyl) at 3333 cm^{-1} on the chalcone compound 8 was disappeared. Other characteristic vibration peaks for phthalonitrile derivative (8a) were observed aromatic C–H stretching at 3071 cm^{-1}, aliphatic C–H stretching at 2965-2855; carbonyl groups (C=O) at 1664 cm^{-1}, Ar–C=C group stretching at 1578-1436 cm-1 and ether group (Ar–O–Ar) stretching at 1385-1246 cm^{-1}.

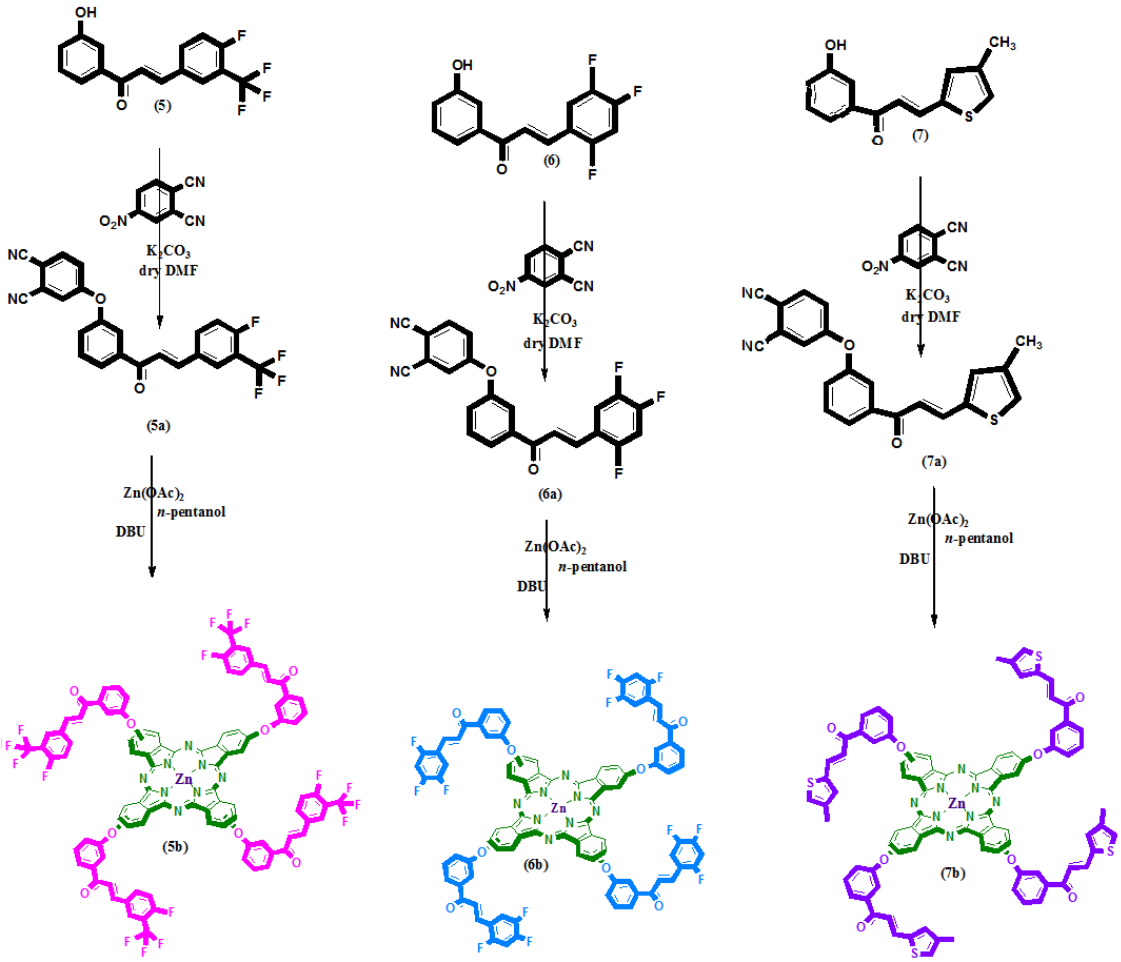

Figure 3. The synthetic route to the phthalonitrile compounds (5a [37], 6a [39], 7a [38]) and zinc phthalocyanine (5b [37], 6b [39], 7b [38])

In the IR spectrum of zinc phthalocyanine (ZnPc), no nitrile (C≡N) vibration band at 2232 cm^{-1} is considered as a clue of the formation of ZnPc (8b). The remaning IR stretching vibrations of (8b) were akin to the phthalonitrile compound (8a) (Figure 4).

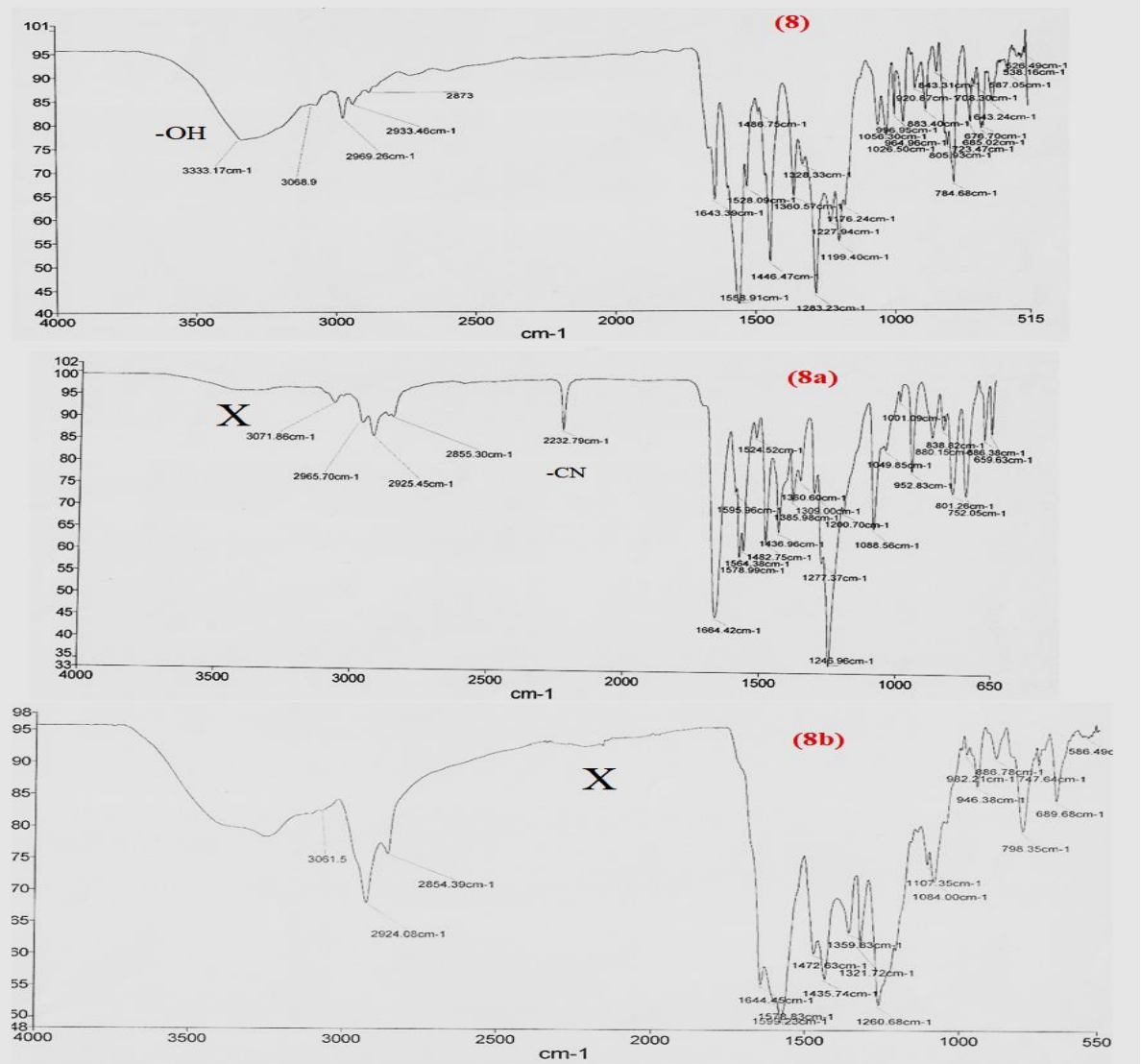

Figure 4. FT-IR spectra of original chalcone (8), phthalonitrile (8a) and zinc phthalocyanine (8b) compounds

In ^{1}H NMR spectrum of chalcone compound (8), phenolic OH signal was observed at 6.57 ppm together with the aromatic and aliphatic CH signals (Figure 5a). The C=O group was detected in the ^{13}C NMR spectrum, at 190.20 ppm, is evidence of the formation of the target structure (8) (Figure 5b). In the ^{1}H NMR spectrum of the phthalonitrile compound (8a), the peak for OH group at 6.57 ppm was disappeared and that for aromatic and aliphatic protons were observed at around 8.03-7.33 ppm and 2.65-1.27 ppm, respectively. ^{13}C NMR spectrum of 8a exhibited carbonyl carbon atom (C=O) at 196.52 ppm, nitrile carbon atoms (C≡N) at 117.86 and 115.20 ppm, aromatic carbon atoms between at 162.55 ppm and 108.50 ppm, and aliphatic carbon atoms (–CH_2 and –CH_3) 31.44 ppm and 26.71 ppm (SI Figure 1 in Supplementary Information). In the ^{1}H NMR spectrum of zinc(II) phthalocyanine complex (8b) exhibited aromatic protons at 8.32 (s, 44H, Ar–H) and aliphatic protons at 3.39 (s, 8H, –CH_2), 1.62 (m, 12H, –CH_3).

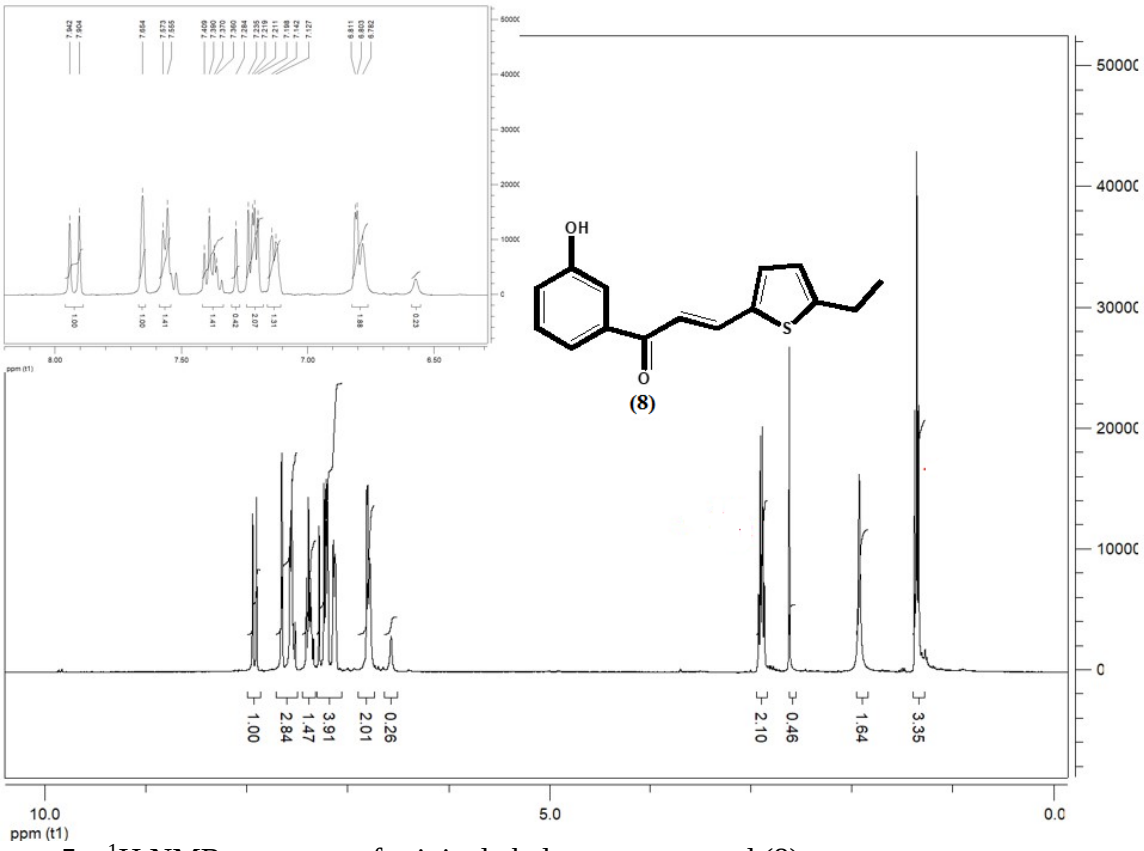

Figure 5a. ^{1}H NMR spectrum of original chalcone compound (8)

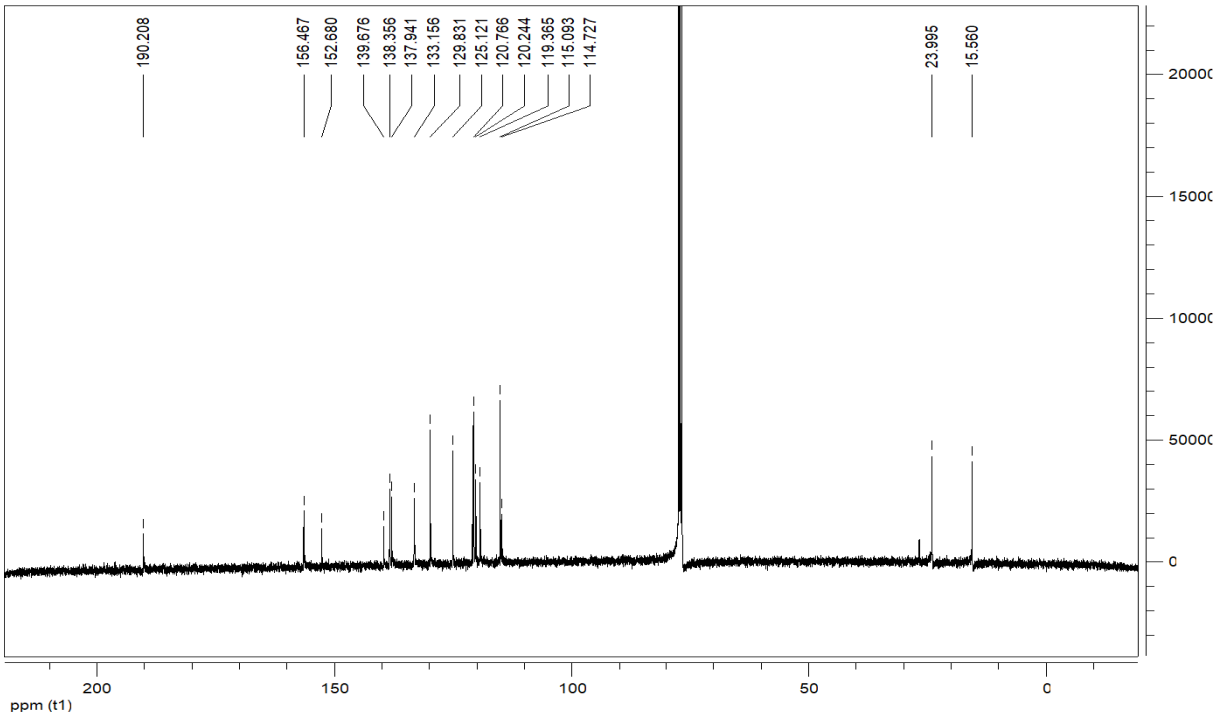

Figure 5b. ^{13}C NMR spectrum of original chalcone compound (8).

In the MALDI-TOF-MS spectra (Figure 6a and 6b), the molecular ion peaks of chalcone (8) and phthalonitrile (8a) were observed at 258.81 $[M]^+$ and 381.13 $[M-3]^+$, respectively. But the molecular ion peak of ZnPc (8b) was not detected due to non-ionisation.

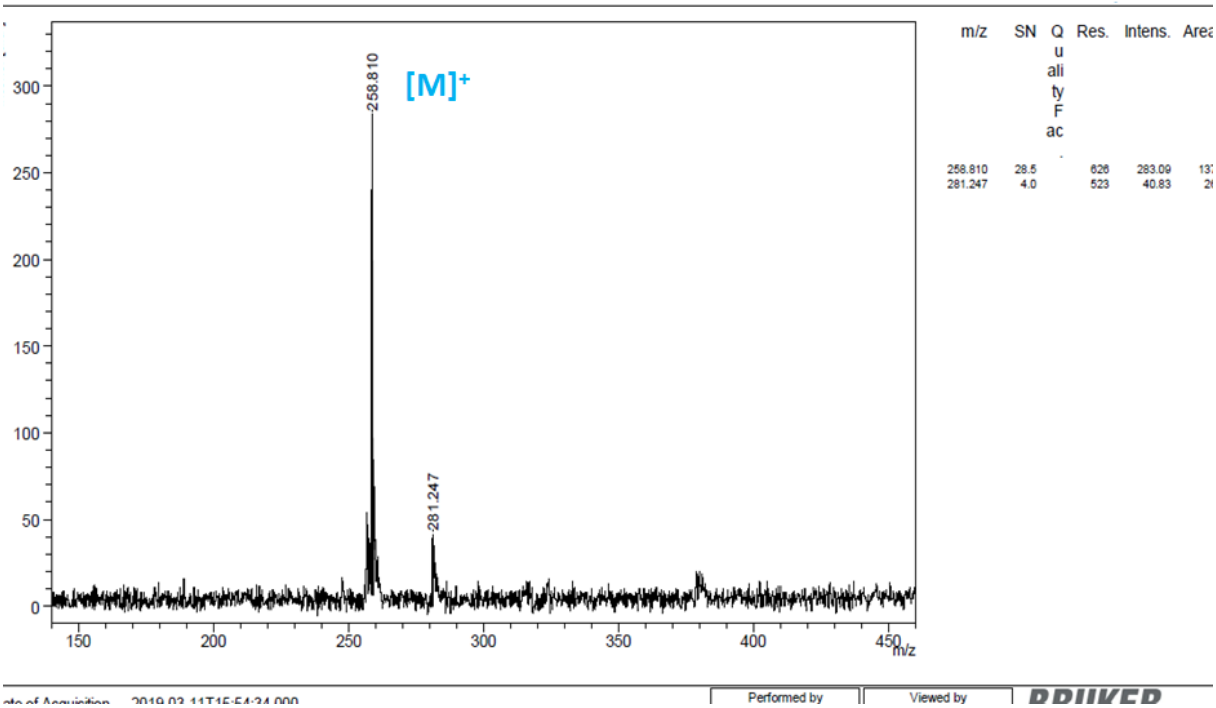

Figure 6a. MALDI-TOF-MS spectra of the chalcone compound (8)

As seen in our previous study [11,37,38,39], many fragmentation products peak are seen in the mass spectrum of phthalonitrile compounds containing chalcone (Figure 6b).

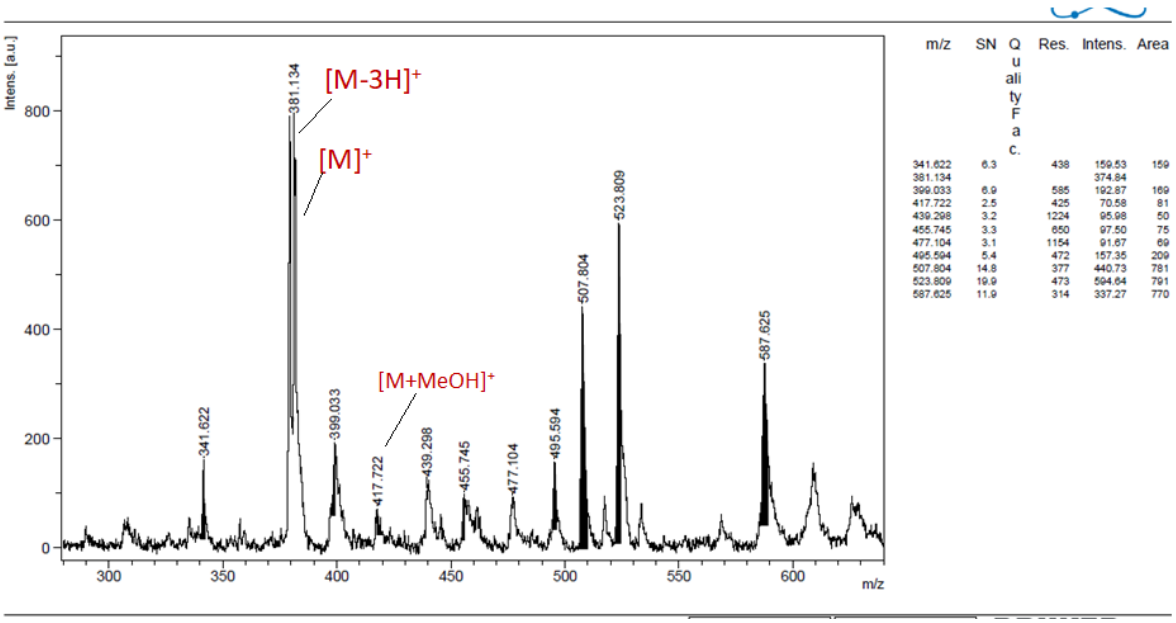

Figure 6b. MALDI-TOF-MS spectra of the phthalonitrile derivative bearing chalcone group (8a).

3.2. UV-Vis absorption spectra and aggregation properties of ZnPc (8b)

Since phthalocyanines are rich in π electrons, they give characteristic absorption bands corresponding to n-π* or π-π* electronic transitions in the ultraviolet and visible region [25]. The UV-vis spectra of phthalocyanines in solution display two strong characteristic absorption bands in the UV-visible (UV-Vis) region. The first is the Soret Band (π-π*) at 300-500 nm in the near UV, while the second is the Q Band (n-π*) at 600-700 nm in the red region of the spectrum [33]. It is important to investigate the absorption properties of phthalocyanines to determine their usability in many applications.

The aggregation depends on the type of solvent, metal ions in the core, pH of the solution, concentration, temperature and the structures of the substituents [25]. In this study, the aggregation behavior of ZnPc (8b) was investigated in different solvents in the UV-Vis spectroscopy (Figure 7) (Table 1).

Table 1. Electronic spectral data for the ZnPc (8b) in various solvents at 1×10^{-5} M.

Zinc Phthalocyanine **(8b)**		
Solvents	**Q band , λ_{max} (nm)**	**(log ε)**
$CHCl_3$	685 /616	(4.97) /(4.62)
DCM	683 /615	(5.02) /(4.61)
DMF	681 /613	(5.05) /(4.69)
DMSO	683 /615	(5.05) /(4.78)

The absorption intensities of Q band vary markedly by the solvent. Therefore, it is important to examine the aggregation behavior of the ZnPc (8b) in assorted solvents such as DMSO, DMF, chloroform ($CHCl_3$) and dichloromethane (DCM). The effects of these solvents on the aggregation of ZnPc (8b) were shown in Figure 7. ZnPc (8b) demonstrated the highest absorbance value in $CHCl_3$ and the lowest absorbance value in DMF.

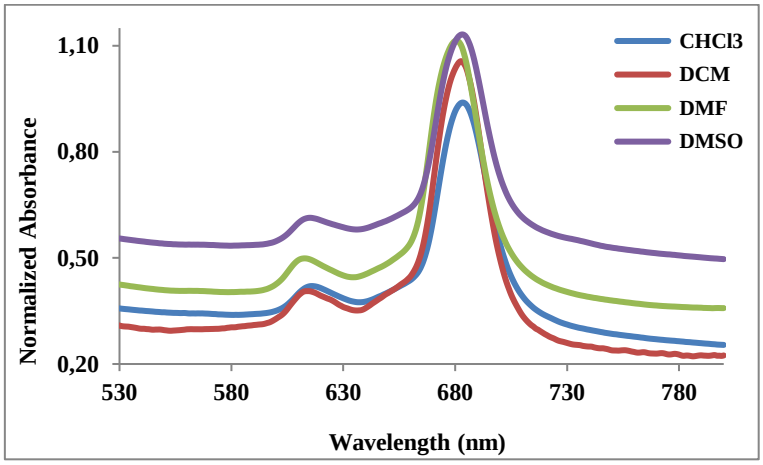

Figure 7. UV-Vis spectrum of zinc phthalocyanine compound (8b)

3.3. Leishmanicidal Activity Results

Leishmanicidal activities of compounds (5-8; 5a-8a; 5b-8b except 7b) against *Leishmania infantum* and *Leishmania major* promastigotes were ascertained *in vitro* by microdilution broth assay with alamar blue. Chalcone compounds (5-8) were found to be efficient at different concentrations (LC: 156-312 µg/ml). While the chalcone-derived phthalonitrile compounds except 7a and 8a showed activity at high concentrations, zinc phthalocyanine compounds (5b, 6b and 8b) bearing different chalcone group did show no activity at the concentrations studied (Figures 8- 9, and Table 2).

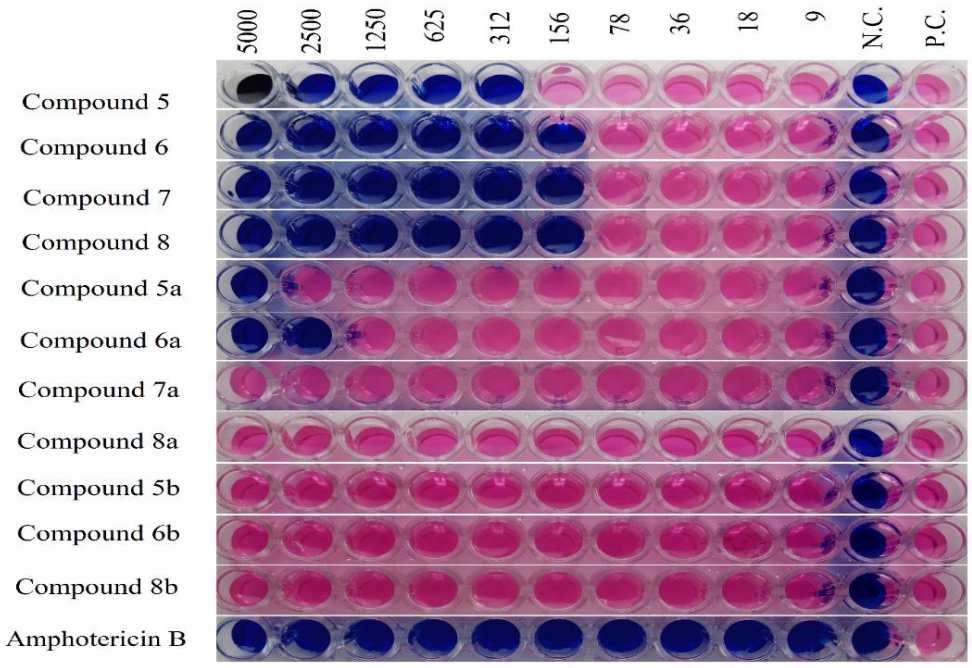

Figure 8. *In vitro* leishmanicidal activity results of compounds.

It is seen in the literature that chalcone compounds with different chemical structures have been studied at different concentrations and with different Leishmania species by different methods [40,41]. It would not be correct to compare the Leishmania activity results of this study with

literature. While IC_{50} was studied in most articles [40,41], in our study the full leishmanicidal activity was studied (it can be called IC_{100}). There should be no living parasites. In addition, the concentrations of the compounds were calculated as microgram/milliliter (µg/mL) and dilution was made accordingly. Among the lesimania activity studies in the literature conducted with the same method, it was observed that the lesimania activities of the chalcone derivatives synthesized were active compared to other chemical structures [42,43].

Table 2. The Leishmanicidal Concentration (LC) values

Compounds	**LC value for** *Leishmania* ***infantum*** **(µg/mL)**	**LC value for** *Leishmania* ***major*** **(µg/mL)**
(5)	312	156
(6)	156	156
(7)	156	156
(8)	156	312
(5a)	5000	5000
(6a)	2500	2500
(7a)	>5000	>5000
(8a)	>5000	>5000
(5b)	>5000	>5000
(6b)	>5000	>5000
(8b)	>5000	>5000
Amphotericin B	<9	<9

It was found that amphotericin B drug used as a standard has leishmanicidal activity against *Leishmania infantum* and *Leishmania major* at all concentrations (LC: <9 µg/ml). It was determined that there was a perfect agreement between the color change of the indicator dye alamar blue in the wells and the microscopy results. In the evaluation of leishmanicial activity, the color of alamar blue remained unchanged in the negative control. In the positive control, the blue color turned pink as the vitality continued. The LC values obtained from the tests were given in Table 2 (Figures 8 and 9).

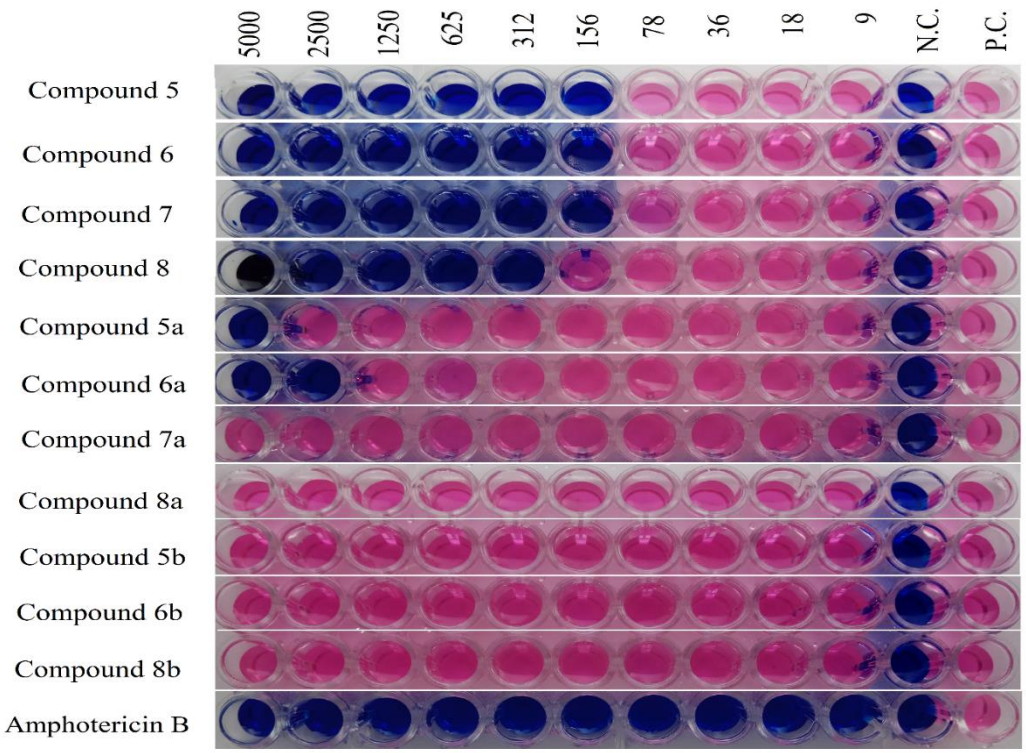

Figure 9. *In vitro* leishmanicidal activity results

In order for chalcone series compounds to be good drug nominee, *in vivo* toxicity tests and control studies should be performed in experimental animal models [44].

4. Conclusion

In this study, it was aimed to study the *in vitro* leishmanicidal activity of different chalcone derivatives and their phthalonitrile and phthalocyanine compounds. In accordance with this purpose, we synthesized different chalcone (5, 6, 7 and 8), phthalonitrile (5a, 6a, 7a and 8a) and zinc(II) (5b, 6b, 7b and 8b) phthalocyanine compounds substituted with chalcone compound. The synthesized novel chalcone compound (8), phthalonitrile (8a) and zinc phthalocyanine derivative (8b) were characterized by merger of the IR, ^{1}H and ^{13}C NMR, UV-Vis and MALDI-TOF-MS spectroscopies. Antiparasitic activities of the compounds (5-8, 5a-8a, 5b, 6b and 8b) were tested as *in vitro* against *Leishmania infantum* and *Leishmania major* promastigotes by Microdilution broth method. The leishmanicidal activities of the compounds were assessed by considering their LC values and the results obtained were contrasted with the results of the standard control drug Amphotericin B. In conclusion, the antiparasitic activity results of the chalcone compounds (5-8) synthesized in the study are promising, and due to their *in vitro* leishmanicidal activities, especially chalcone series compounds can be evaluated in pharmacological studies and the diversity of compounds may increase. In order for chalcone

series compounds to be good drug nominee, *in vivo* toxicity tests and control studies should be performed in experimental animal models. It is essential to multiply such studies in terms of combating this disease for which there is no effective vaccine.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

- Ayşe AKTAŞ KAMİLOĞLU: Designing the study, synthesis of organic and inorganic compounds, analysis, evaluation of results, writing the article
- Şahin DİREKEL: Performing leishmanicidal activity tests, evaluation of results, writing the article
- Gonca ÇELİK: Synthesis of organic compounds

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